Metabolic network analysis and regulation

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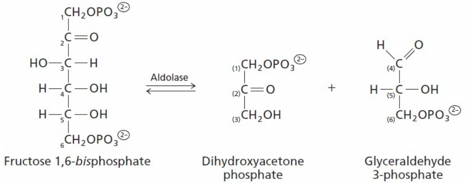
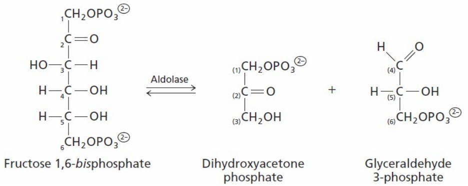
Metabolism



A biochemical reaction

Metabolism



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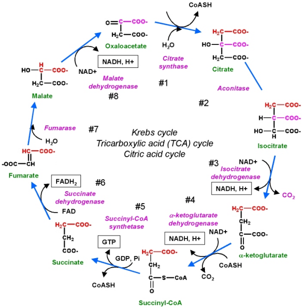
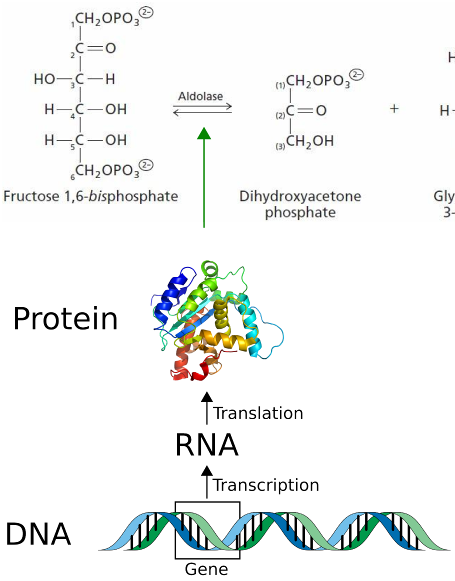
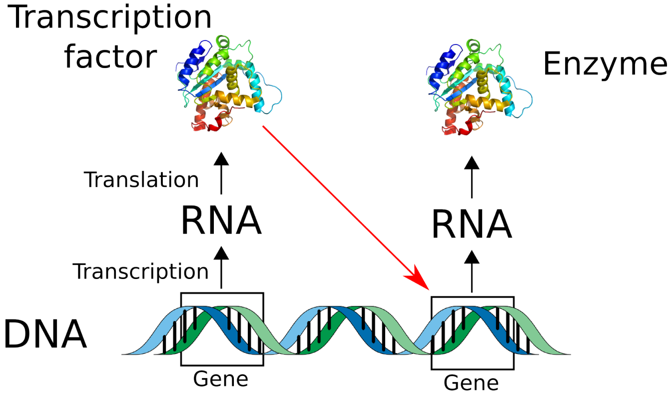


Example of a metabolic pathway : The Krebs cycle

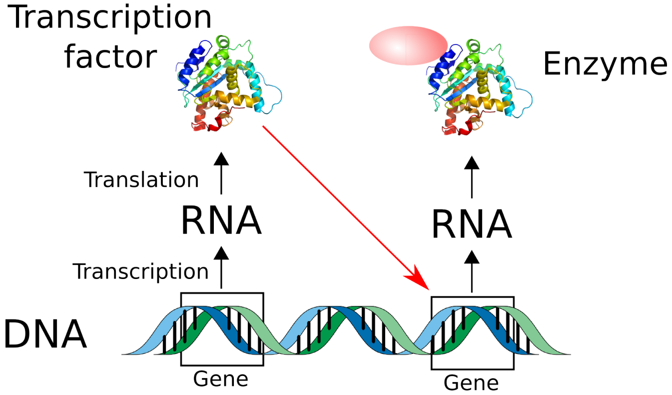
Metabolism


Fructose 1,6-bisphosphate
Aldolase
Dihydroxyacetone phosphate
Glyceraldehyde 3-phosphate
Protein
Translation
RNA
Transcription
DNA
Gene

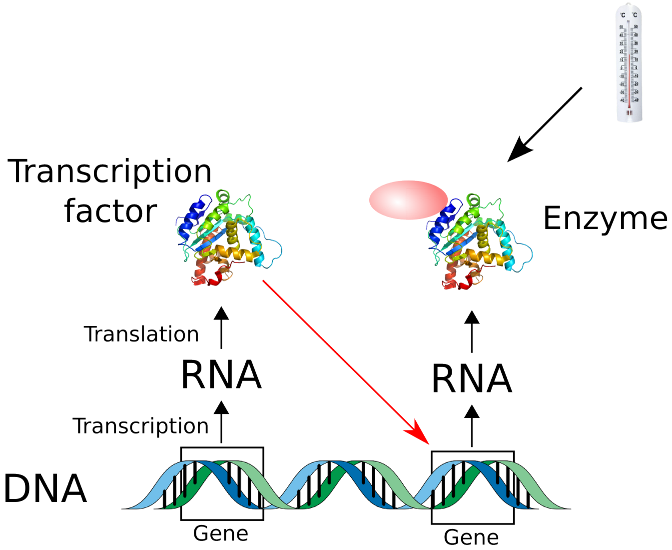
Metabolism regulation



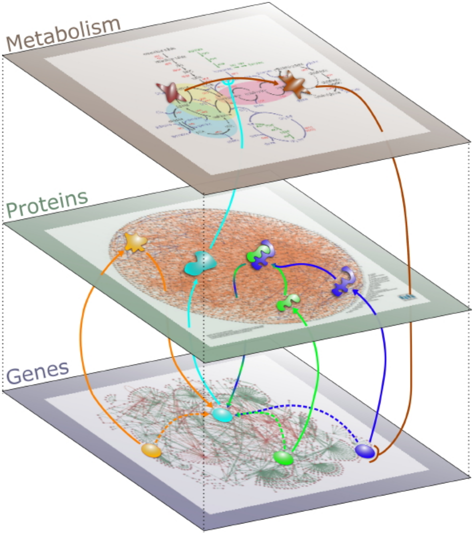
Metabolism regulation



Metabolism regulation

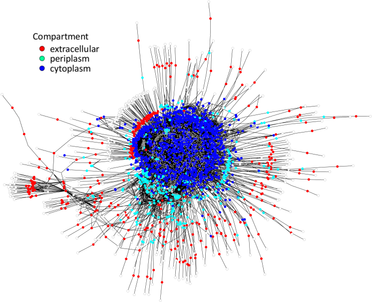


Biological networks

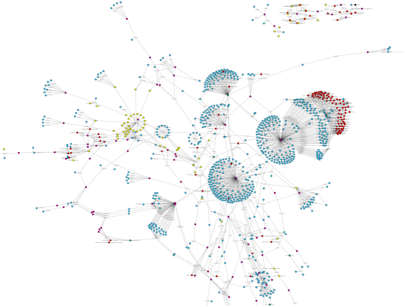


Biological context

Ralstonia solanacearum
With Ludovic Cottret and Remi Peyraud



Reconstructed metabolic network.

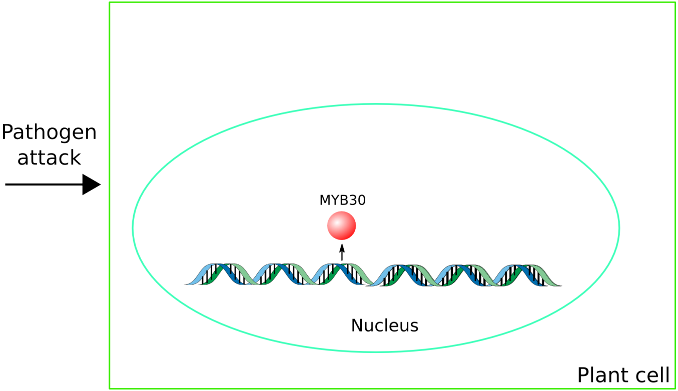


Reconstructed virulence regulatory network.

Biological context

Arabidopsis Thaliana defence response

Supervisors : Susana Rivas and Philippe Besse



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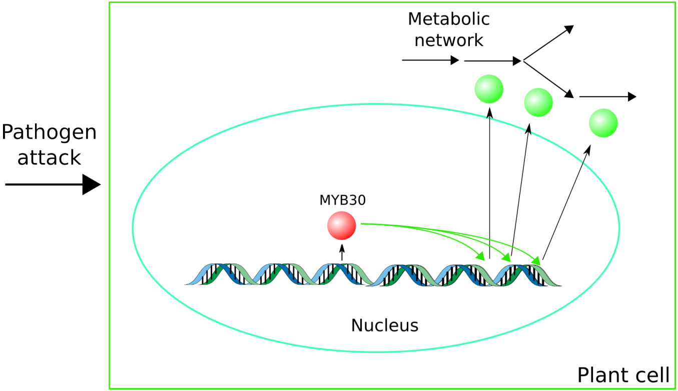

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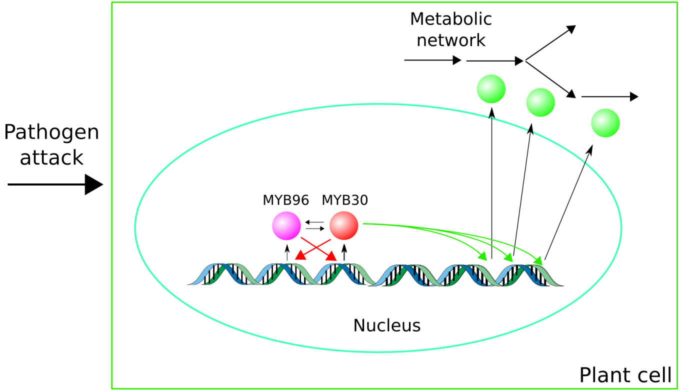

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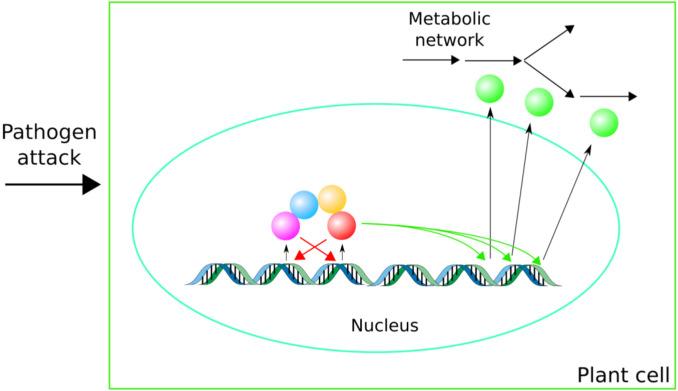
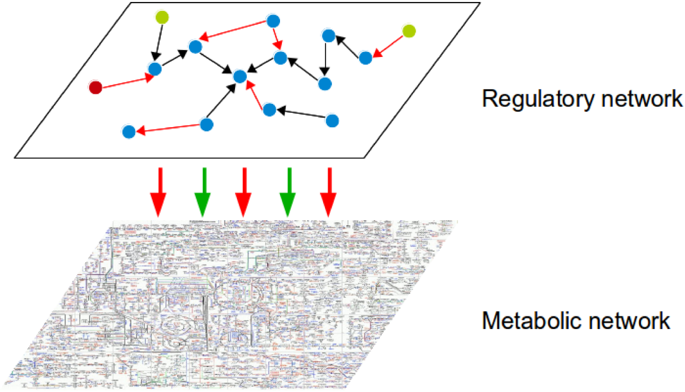
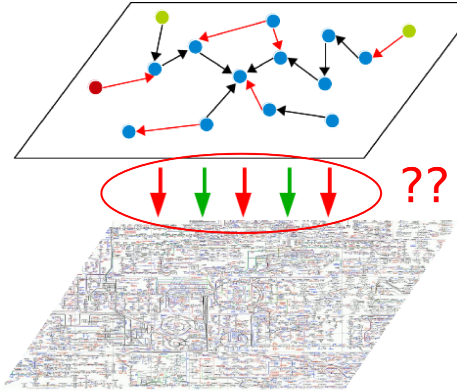



Regulatory network
Metabolic network

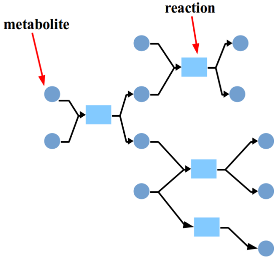


Regulatory network

Metabolic network

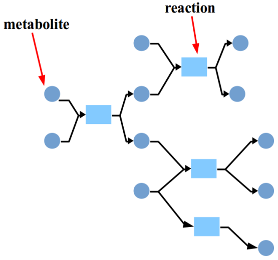
Metabolic network analysis

Graph theory



Metabolic network analysis

Graph theory



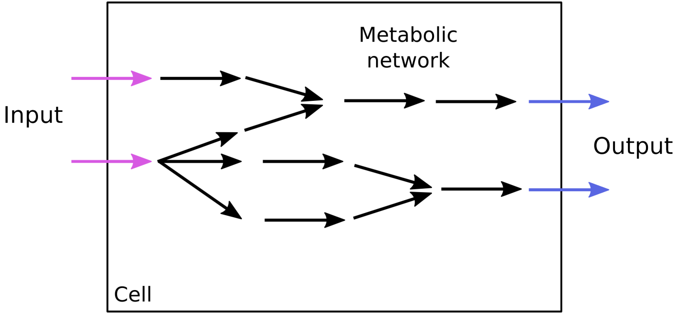
Kinetic parameters

$$A+B \underset{k2}{\overset{k1}{\rightleftharpoons}} C \qquad \frac{d[C]}{dt} = k1[A][B]-k2[C]$$

Metabolic network analysis

Constraint based method : Flux Balance Analysis (FBA)

- Allows to calculate the fluxes (rates) of the reactions of the metabolic network at steady state.



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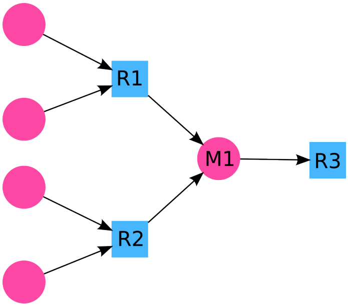

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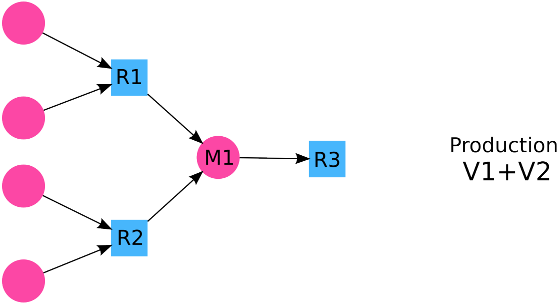

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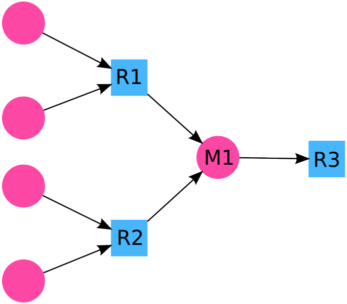

Production

$$V1+V2 - V3 = 0 \text{ mmol/g/h}$$

Consumption

Metabolic network analysis

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R1 : A_ext → A
R2 : A → B
R3 : A → C
R4 : B + E → 2D
R5 : E_ext → E
R6 : 2B → C + F
R7 : C → D
R8 : D → D_ext
R9 : F → F_ext

S

	R1	R2	R3	R4	R5	R6	R7	R8	R9
A	1	-1	-1						
B		1		-1		-2			
C			1			1	-1		
D				2			1	-1	
E				-1	1				
F						1			-1

http://bio.freelogy.org/wiki/User:JeremyZucker

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R7 : C → D
R8 : D → D_ext
R9 : F → F_ext

S

	R1	R2	R3	R4	R5	R6	R7	R8	R9
A	1	-1	-1						
B		1		-1		-2			
C			1			1	-1		
D				2			1	-1	
E				-1	1				
F						1			-1

X

V
v1
v2
v3
v4
v5
v6
v7
v8
v9

Metabolic network analysis

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R7 : C → D
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	R1	R2	R3	R4	R5	R6	R7	R8	R9
A	1	-1	-1						
B		1		-1		-2			
C			1			1	-1		
D				2			1	-1	
E				-1	1				
F						1			-1

X

v
v1
v2
v3
v4
v5
v6
v7
v8
v9

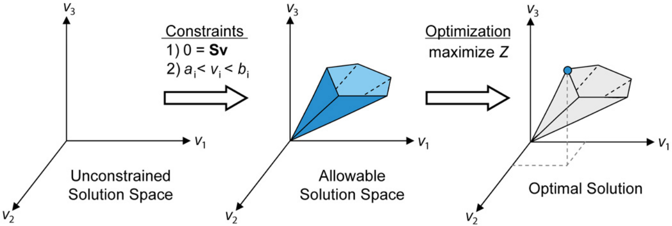
S.v = 0

dA/dt = v1 – v2 – v3 = 0
dB/dt = v2 – v4 - 2*v6 = 0
dC/dt = v3 + v6 – v7 = 0
dD/dt = 2*v4 + v7 – v8 = 0
dE/dt = -v4 + v5 = 0
dF/dt = v6 – v9 = 0

Metabolic network analysis

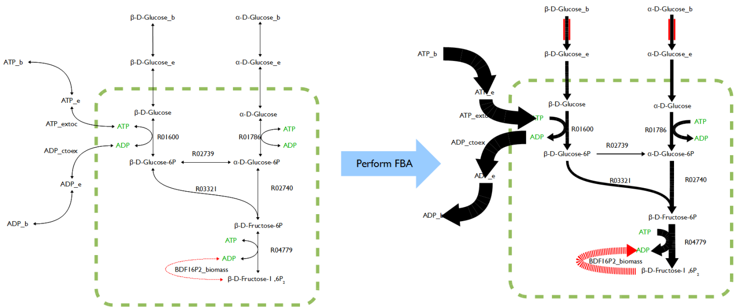
Méthode basée sur des contraintes : Analyse d'équilibre des flux (FBA)

- Allows to calculate the fluxes (rates) of the reactions of the metabolic network at steady state.
- Based on the stoichiometry of the network and constraints on the input fluxes. linéaires).
- Resolves a system of linear equations with an objective function.

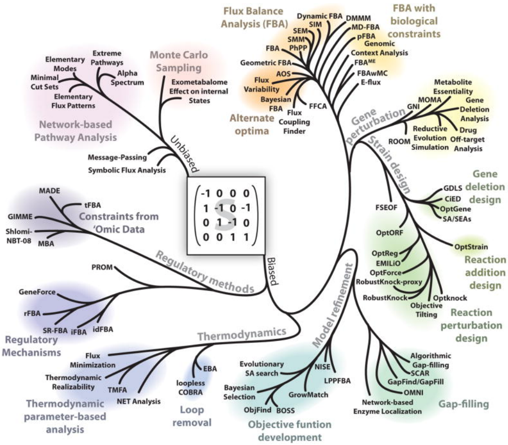


Orth *et al.* 2011

Metabolic network analysis

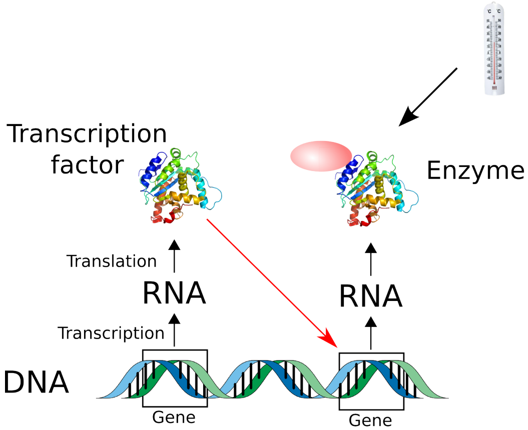


Constraint based methods phylogeny

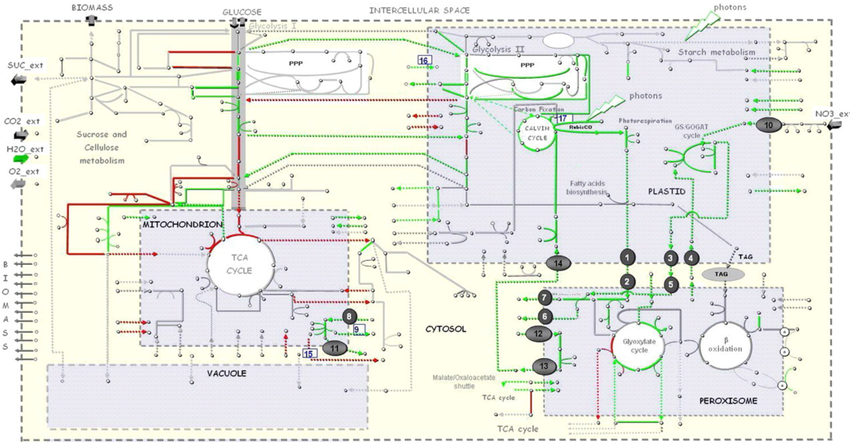


Lewis *et al.* 2013

Metabolic network regulation

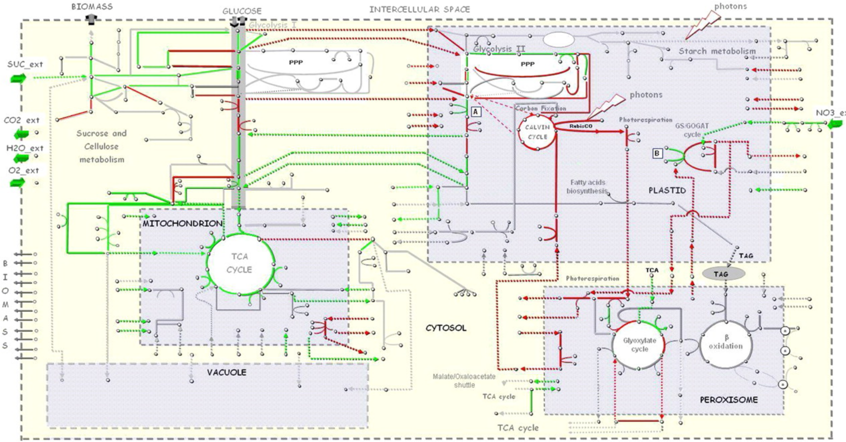


Metabolic network regulation



de Oliveira Dal’Molin *et al.* 2010

Metabolic network regulation



de Oliveira Dal'Molin *et al.* 2010

Regulatory network

Nature

- Composed of biological entities of different types that interact in different ways.
- Cause a specialisation (reversible or not) of the metabolism.

Analyses and simulations

- Many methods (de Jong *et al.* 2002, Karlebach *et al.* 2008).

Regulatory network logical modelling

Boolean model

- Each component has only two possible states.
- The value of each component is determined by a logical function.

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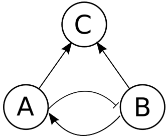

$$f_A(\mathrm{B}) = \mathrm{B}$$
$$f_B(\mathrm{A}) = \mathrm{NOT\ A}$$
$$f_C(\mathrm{A,B}) = \mathrm{A\ AND\ B}$$

Regulatory network logical modelling

Multi-state model

- Each component has a finite number of possible states.
- Each component has a list of rules to determine its state and a default value.

Regulatory network logical modelling

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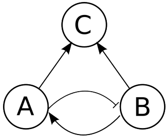

A

Condition	Value
B=1	1
Default	0

B

Condition	Value
A=1	0
Default	1

C

Condition	Value
A=1 XOR B=1	1
A=1 AND B=1	2
Default	0

Regulatory network logical modelling

Simulations

- Update of the network states to find steady states.

A

Condition	Value
B=1	1
Default	0

B

Condition	Value
A=1	0
Default	1

C

Condition	Value
A=1 XOR B=1	1
A=1 AND B=1	2
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	T0	T1	T2	T3	T4
A	0				
B	1				
C	0				

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	T0	T1	T2	T3	T4
A	0	1			
B	1	1			
C	0	1			

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	T0	T1	T2	T3	T4
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B	1	1	0		
C	0	1	2		

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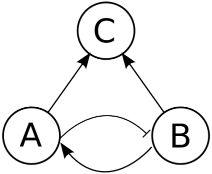
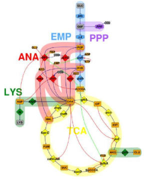
How to combine regulatory network analysis and FBA ?

Logical regulatory network



Qualitative values

FBA



Quantitative values

How to combine regulatory network analysis and FBA ?

Regulatory network steady state		FBA constraint
R1 = 0	$\longrightarrow$	a < R1 < b
R2 = 1		c < R2 < c

Constrain the FBA with regulatory network states

- Find a steady state of the regulatory network, constrain the reactions and perform a FBA analysis.

How to combine regulatory network analysis and FBA ?

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Constrain the FBA with regulatory network states

- Find a steady state of the regulatory network, constrain the reactions and perform a FBA analysis.
- What network state to use ?

	T0	T1	T2	T3	T4
A	0	1	1	0	0
B	1	1	0	0	1
C	0	1	2	1	0

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- How to transform a qualitative state into a constraint ?

Qualitative state to constraint translation

Regulatory network steady state		FBA constraint
$R1 = 0$	$\longrightarrow$	$a < R1 < b$
$R2 = 1$		$c < R2 < c$

Qualitative state to constraint translation

Regulatory network steady state		FBA constraint
R1 = 0 R2 = 1	$\longrightarrow$	a < R1 < b c < R2 < c

Regulatory network state		FBA constraint
R1 = 0 R2 = 1	$\longrightarrow$	0 < R1 < 0 -inf < R2 < +inf

Qualitative state to constraint translation

Regulatory network steady state		FBA constraint
R1 = 0	$\longrightarrow$	a < R1 < b
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Regulatory network state		FBA constraint
R1 = 0	$\longrightarrow$	0 < R1 < 0
R2 = 1		-inf < R2 < +inf

Regulatory network state		FBA constraint
R1 = 0	$\longrightarrow$	0 < R1 < 1
R2 = 2		3 < R2 < 5

R2	
0	[-inf,0]
1	]0,3]
2	]3,5]
3	]5,+inf]

What network state to use ?

	T0	T1	T2	T3	T4
A	0	1	1	0	0
B	1	1	0	0	1
C	0	1	2	1	0

What network state to use ?

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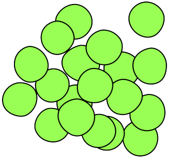

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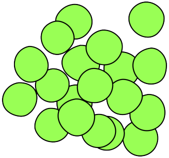


C	
0	[0,0]
1	]0,5]
2	]5,+inf]

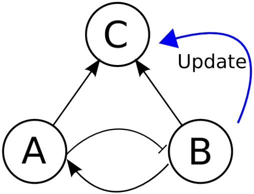
0 < C < 0
0 < C < 5
5 < C < +inf <=> 5/4 < C < +inf
0 < C < 5

How to combine regulatory network analysis and FBA ?

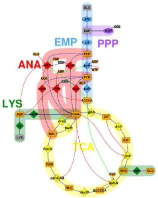
Dynamic analysis

- Metabolite concentrations allow to go back from FBA results to a regulatory network state.

Logical regulatory network



FBA



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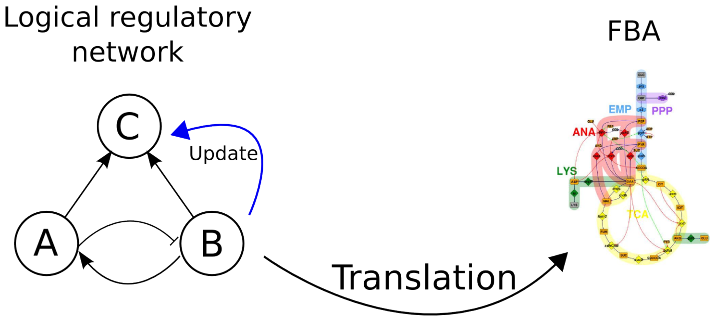

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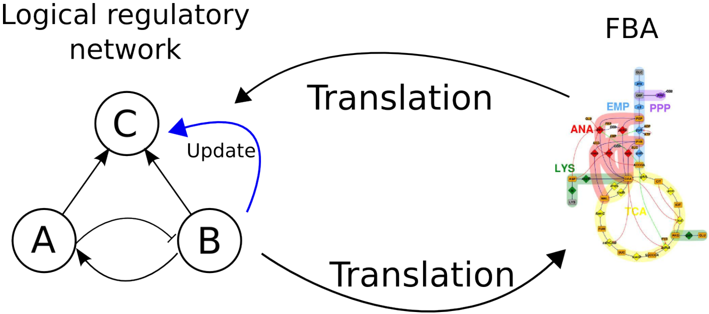

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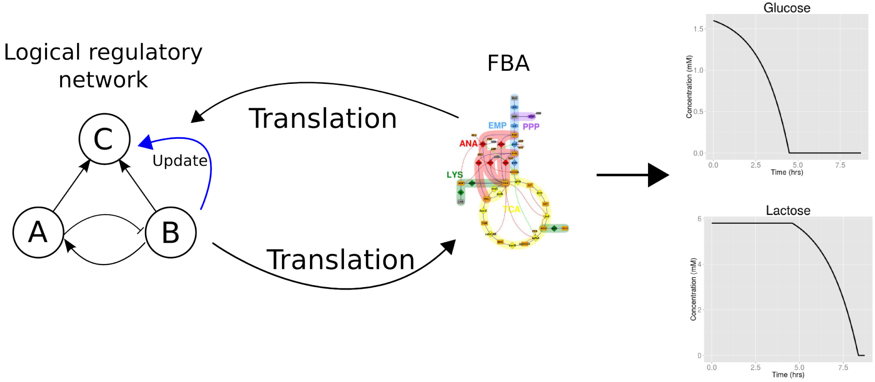


What tools are available for these analyses ?

File formats

- Metabolic network : Systems Biology Markup Language (SBML). (Hucka *et al.* 2003)
- Regulatory network : SBML qualitative extension. (Chaouiya *et al.* 2013)

Software

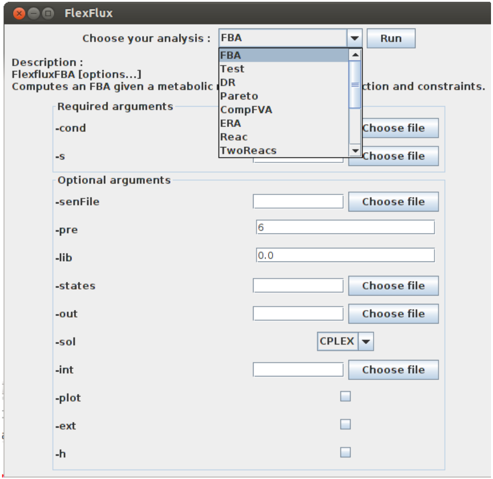
- FBA : Cobra toolbox, surrey FBA, optFlux ...
- Boolean regulatory network : Boolnet.
- Multi-state regulatory network : GINsim, The Cell Collective.

FlexFlux

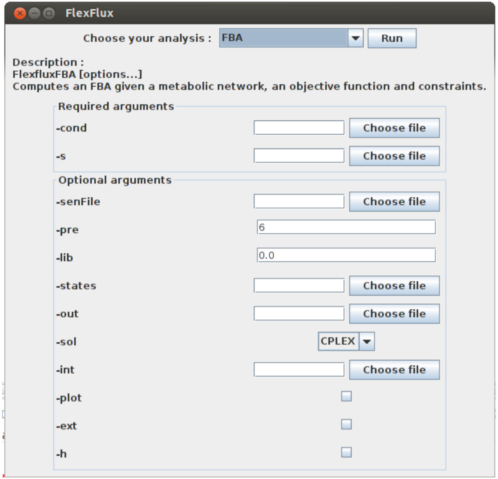
Java software

- Allows analysis of both metabolic and regulatory networks.
- Supports SBML and SBML-qual file formats.
- Features steady state and dynamic analysis of the regulatory network.
- Features multiple FBA based functions.
- Documentation and examples available at `http://lipm-bioinfo.toulouse.inra.fr/flexflux/`

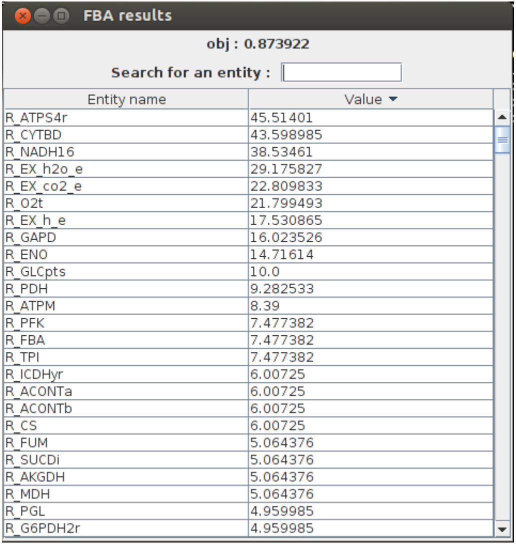
FlexFlux interface



FlexFlux interface



FlexFlux interface



FBA results

obj : 0.873922

Search for an entity :

Entity name	Value
R_ATPS4r	45.51401
R_CYTBD	43.598985
R_NADH16	38.53461
R_EX_h2o_e	29.175827
R_EX_co2_e	22.809833
R_O2t	21.799493
R_EX_h_e	17.530865
R_GAPD	16.023526
R_ENO	14.71614
R_GLCpts	10.0
R_PDH	9.282533
R_ATPM	8.39
R_PFK	7.477382
R_FBA	7.477382
R_TPI	7.477382
R_ICDHyr	6.00725
R_ACONTa	6.00725
R_ACONTb	6.00725
R_CS	6.00725
R_FUM	5.064376
R_SUCDi	5.064376
R_AKGDH	5.064376
R_MDH	5.064376
R_PGL	4.959985
R_G6PDH2r	4.959985

FlexFlux interface

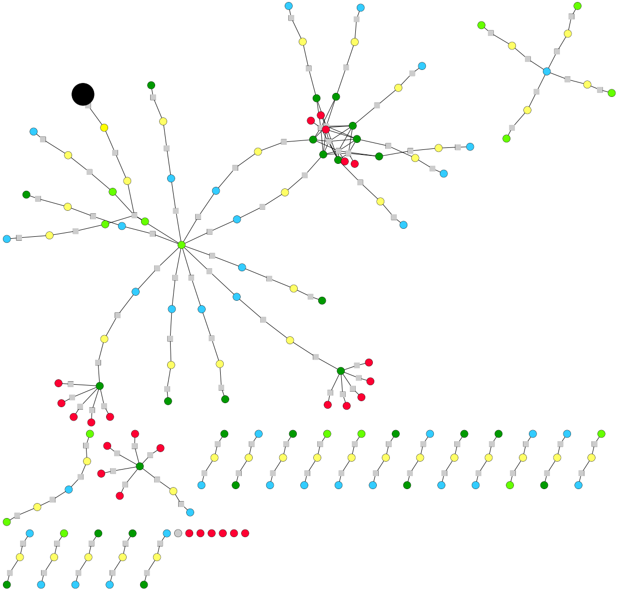
Steady state analysis results

Search for an entity :

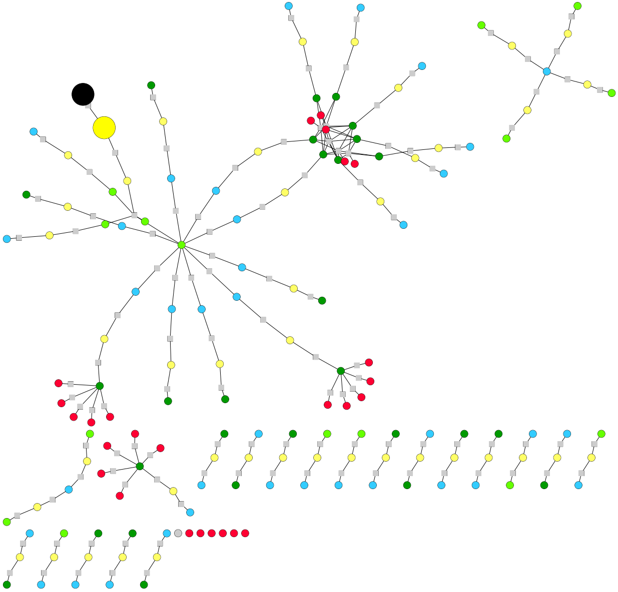
Attractor of size 1

Entity n...	1	2	3	4	5	6	7	8
KCS16_p	0	0	0	0	0	0	0	0
HCD1	0	0	0	0	1	1	1	1
KCS3_m...	0	0	0	0	0	0	0	0
KCS5_m...	0	0	0	0	0	0	0	0
CER4	0	0	0	0	1	1	1	1
KCS1_p	0	0	0	0	0	0	1	1
MIEL4_p3	0	0	0	0	0	0	0	0
sPLA2_a...	0	0	0	0	0	0	0	0
MIEL4_m...	0	0	0	0	0	0	0	0
MIEL3_m...	0	0	0	0	0	0	0	0
KCS4_p	0	0	0	0	0	0	0	0
MYB31_p	0	0	0	0	0	0	0	0
MIEL2_p	0	0	0	0	0	0	0	0
SBT52_p2	0	0	0	0	0	0	0	0
HCD1_p	0	0	0	0	0	0	1	1
MYB30	0	1	1	1	1	1	1	1
KCS11_p	0	0	0	0	0	0	0	0
MYB30_...	0	0	1	1	1	1	1	1
sPLA2_a...	0	0	0	0	0	0	0	0
MIEL4_p1	0	0	0	0	0	0	0	0
CER4_p	0	0	0	0	0	0	1	1
R_VLCFA...	0	0	0	0	0	0	0	1
MIEL3_p	0	0	0	0	0	0	0	0
KCS10_p	0	0	0	0	0	0	1	1
R_VLCFA...	0	0	0	0	0	0	0	0

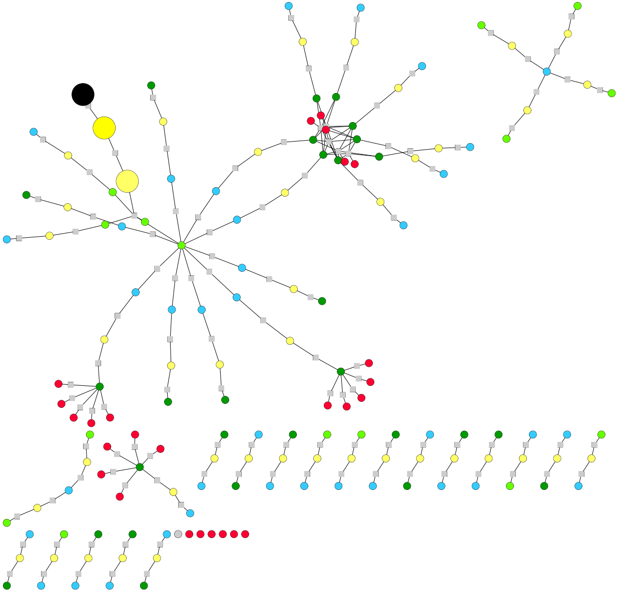
FlexFlux steady state simulations



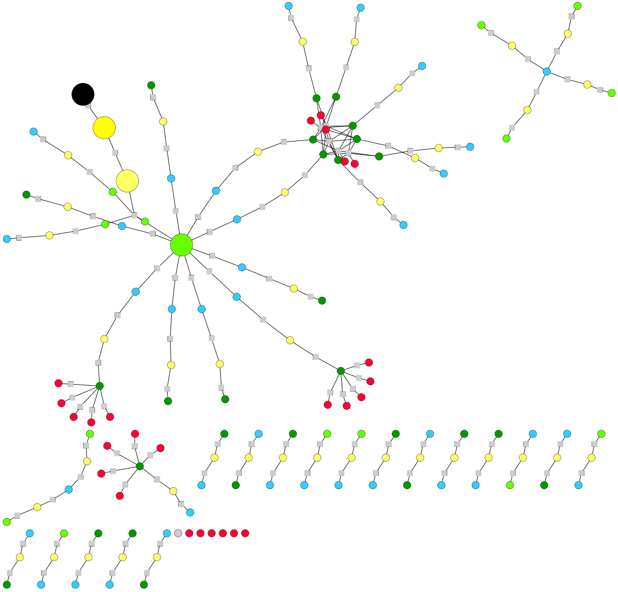
FlexFlux steady state simulations



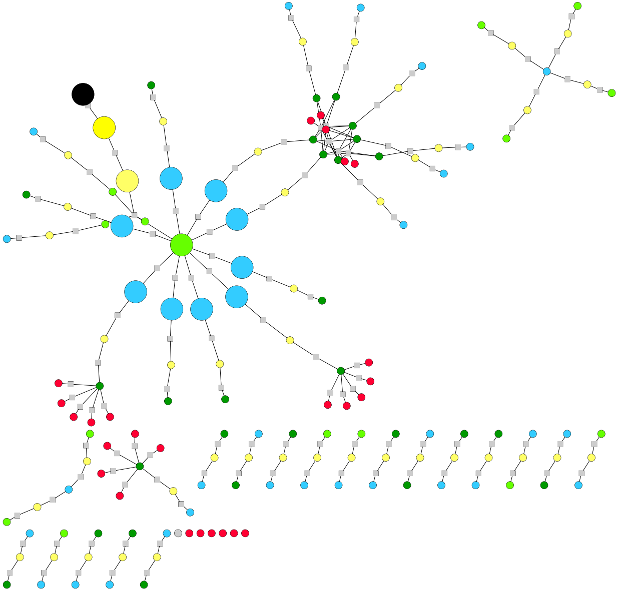
FlexFlux steady state simulations



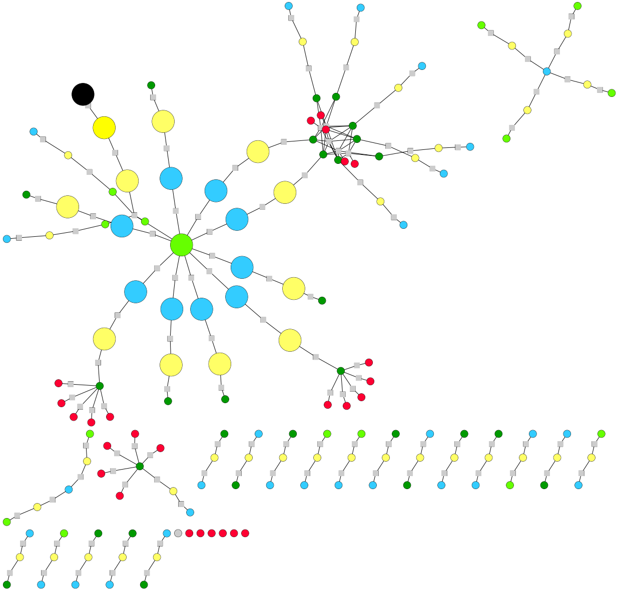
FlexFlux steady state simulations



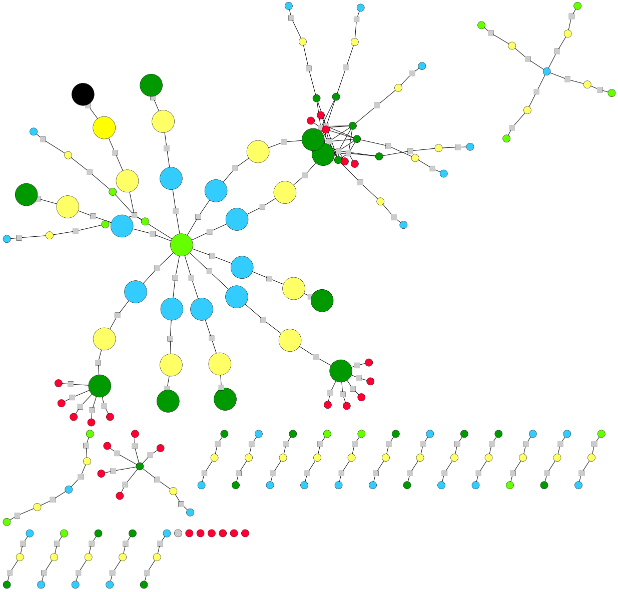
FlexFlux steady state simulations



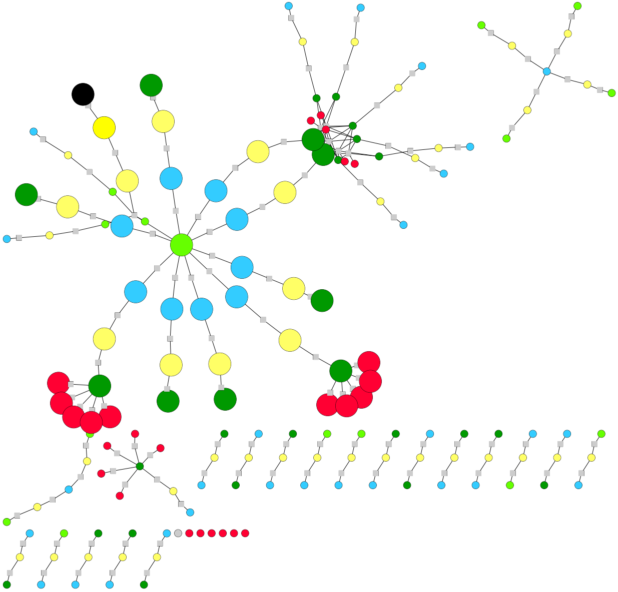
FlexFlux steady state simulations



FlexFlux steady state simulations



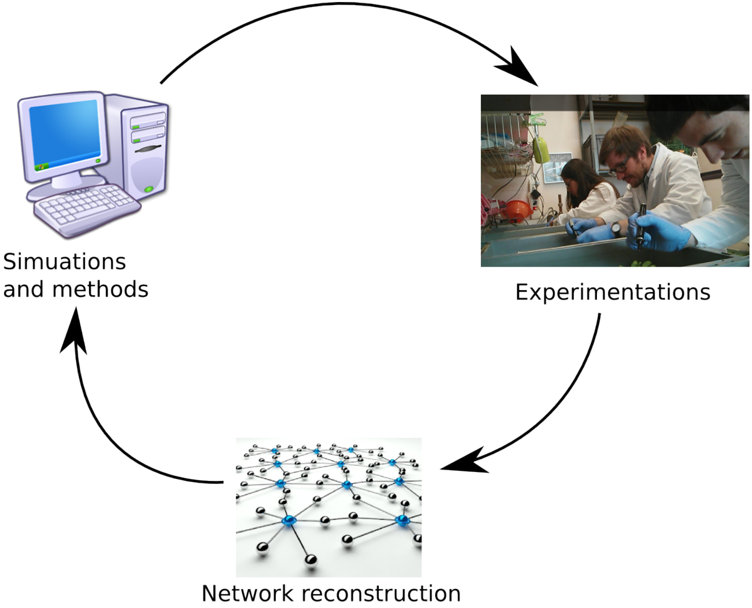
FlexFlux steady state simulations



Conclusion

- Metabolic network analyses are very insightful to understand cell phenotype.
- Metabolism regulation must be included to get a full picture of cell behaviour.
- These two types of networks can be very large and are of a different nature.
- FBA and logical regulatory network modelling provide interesting answers.

Conclusion


Simuations
and methods
Experimentations
Network reconstruction